

AUG 19 2005

AMENDMENT AND RESPONSE UNDER 37 CFR § 1.111

Serial Number 10/684,554

Filing Date: October 12, 2007

Title: ADENO-ASSOCIATED VIRUS VECTORS AND USES THEREOF

Page 2

Dkt: 875.024US1

In the Claims

Please amend the claims as follows:

1. (Currently Amended) A composition comprising at least two recombinant adeno-associated viruses (AAV), comprising:
  - a) a first recombinant AAV comprising a first recombinant DNA molecule comprising linked:
    - i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
    - ii) a second DNA segment which comprises a *cis*-acting heterologous transcriptional regulatory element; and
    - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV; and
  - b) a second recombinant AAV comprising a second recombinant DNA molecule comprising linked:
    - i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
    - ii) a second DNA segment which comprises an entire open reading frame encoding for a therapeutic gene product; and
    - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV,  
wherein the recombinant DNA molecules of the two rAAVs, when contacted with a host cell, become linked, forming a molecule which has the *cis*-acting heterologous transcriptional regulatory element 5' to the open reading frame, wherein the *cis*-acting heterologous transcriptional regulatory element regulates transcriptional expression transcription of the gene product encoded by the open reading frame in a host cell contacted with the first and second rAAVs, wherein if the *cis*-acting heterologous transcriptional regulatory element is an enhancer, transcription of the open reading frame is enhanced by the enhancer, and wherein if the *cis*-acting heterologous transcriptional regulatory element is a promoter, transcription of the open reading frame is initiated at the promoter.
2. (Withdrawn) The composition of claim 1 further comprising a delivery vehicle.

3. (Withdrawn) The composition of claim 2 where the vehicle is a pharmaceutically acceptable carrier.

4-7. (Canceled)

8. (Withdrawn) The composition of claim 1 wherein the second DNA segment of the first recombinant DNA molecule comprises an enhancer.

9. (Previously Presented) The composition of claim 1 wherein the second DNA segment of the first recombinant DNA molecule comprises a heterologous promoter.

10. (Withdrawn) The composition of claim 1 wherein the second DNA segment of the second recombinant DNA molecule comprises the open reading frame but not a heterologous promoter.

11. (Withdrawn) The composition of claim 10 wherein the second DNA segment of the first recombinant DNA molecule comprises a heterologous promoter.

12-18. (Canceled)

19. (Currently Amended) A recombinant adeno-associated viral vector comprising at least one *cis*-acting heterologous transcriptional regulatory element functional in a host cell, which *cis*-acting heterologous transcriptional regulatory element regulates is capable of regulating, in the host cell, transcriptional expression transcription of an entire open reading frame for a therapeutic gene product encoded by a second recombinant adeno-associated viral vector, after sequences in the first and second recombinant adeno-associated virus vectors become linked in the host cell, wherein if the *cis*-acting heterologous transcriptional regulatory element is an enhancer, transcription of the open reading frame is enhanced by the enhancer, and wherein if the *cis*-acting heterologous transcriptional regulatory element is a promoter, transcription of the open reading frame is initiated at the promoter.

20. (Original) The vector of claim 19 wherein the element is a promoter.
21. (Withdrawn) The vector of claim 19 wherein the element is an enhancer.
22. (Canceled)
23. (Withdrawn) A plasmid comprising the vector of claim 19.
24. (Withdrawn) A host cell contacted with the composition of claim 1.
25. (Withdrawn) A host cell contacted with at least two recombinant AAV,  
wherein a first recombinant AAV comprises a first recombinant DNA molecule  
comprising linked:
  - i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
  - ii) a second DNA segment which does not comprise adeno-associated viral  
sequences; and
  - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV;  
andwherein a second recombinant AAV comprises a second recombinant DNA molecule  
comprising linked:
  - i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
  - ii) a second DNA segment which does not comprise adeno-associated viral  
sequences and which second DNA segment is different than the second  
DNA segment of the first recombinant DNA molecule; and
  - iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV.
26. (Withdrawn) A method to transfer recombinant DNAs to a host cell, comprising:  
contacting the host cell with at least two recombinant AAV,

wherein a first recombinant AAV comprises a first recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
- ii) a second DNA segment which does not comprise adeno-associated viral sequences; and
- iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV; and

wherein a second recombinant AAV comprises a second recombinant DNA molecule comprising linked:

- i) a first DNA segment comprising a 5'-inverted terminal repeat of AAV;
- ii) a second DNA segment which does not comprise adeno-associated viral sequences and which second DNA segment is different than the second DNA segment of the first recombinant DNA molecule; and
- iii) a third DNA segment comprising a 3'-inverted terminal repeat of AAV

27. (Withdrawn) A method to transfer and express a polypeptide in a host cell comprising contacting the host cell with the composition of claim 1.

28. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule comprises a portion of an open reading frame operably linked to a promoter.

29. (Withdrawn) The method of claim 28 wherein the first recombinant DNA molecule comprises a splice donor site 3' to the open reading frame.

30. (Withdrawn) The method of claim 29 wherein the second DNA segment of the second recombinant DNA molecule comprises the remainder of the open reading frame which together with the second DNA segment of the first recombinant DNA molecule encodes a full-length polypeptide.

31. (Withdrawn) The method of claim 30 wherein the second DNA segment of the second recombinant DNA molecule comprises a splice acceptor site 5' to the remainder of the open reading frame.
32. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule comprises an enhancer.
33. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule comprises a promoter.
34. (Withdrawn) The method of claim 32 wherein the second DNA segment of the second recombinant DNA molecule comprises at least a portion of an open reading frame.
35. (Withdrawn) The method of claim 33 wherein the second DNA segment of the second recombinant DNA molecule comprises at least a portion of an open reading frame.
36. (Withdrawn) The method of claim 34 wherein the second DNA segment of the second recombinant DNA molecule further comprises a promoter operably linked to the open reading frame.
37. (Withdrawn) The method of claim 35 wherein the second DNA segment of the second recombinant DNA molecule further comprises a promoter operably linked to the open reading frame.
- 38-40. (Canceled)
41. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of the first recombinant DNA molecule further comprises DNA encoding a protein that binds to the origin of replication.

42. (Withdrawn) The method of claim 41 wherein the second DNA segment in the second recombinant DNA molecule comprises a portion of an open reading frame.

43. (Withdrawn) The method of claim 41 wherein the second DNA segment in the second recombinant DNA molecule further comprises a promoter operably linked to the open reading frame.

44-45. (Canceled)

46. (Previously Presented) The composition of claim 1 wherein the second DNA segment of one of the vectors comprises a heterologous transcriptional regulatory element.

47. (Withdrawn) The host cell of claim 25 wherein the second DNA segment of one of the vectors comprises a heterologous transcriptional regulatory element.

48. (Withdrawn) The method of claim 26 or 27 wherein the second DNA segment of one of the vectors comprises a heterologous transcriptional regulatory element.

49. (Withdrawn) A method to enhance the expression of a polynucleotide in a host cell, comprising: contacting a host cell comprising a recombinant AAV vector comprising a polynucleotide segment which encodes a polypeptide, with a composition comprising a further recombinant AAV vector corresponding to the vector of claim 19 in an amount which enhances expression of the polynucleotide.

50. (Withdrawn) A method to enhance the expression of a polynucleotide in a host cell, comprising: contacting a host cell comprising a recombinant AAV vector corresponding to the vector of claim 19, with a composition comprising a further recombinant AAV vector comprising a polynucleotide segment which encodes a polypeptide, in an amount which enhances expression of the polynucleotide.

51. (Withdrawn) A method to enhance the expression of a polynucleotide in a host cell, comprising: contacting a host cell with a recombinant AAV vector corresponding to the vector of claim 19 and a further recombinant AAV vector comprising a polynucleotide segment which encodes a polypeptide, in an amount which enhances expression of the polynucleotide in the cell.

52. (Withdrawn) The method of claim 49 or 50 wherein the composition further comprises a delivery vehicle.

53. (Withdrawn) The method of claim 52 wherein the delivery vehicle is a pharmaceutically acceptable carrier.

54. (Withdrawn) The method of claim 49, 50 or 51 wherein heterologous transcriptional regulatory element in the recombinant AAV corresponding to the vector of claim 19 is a promoter.

55-57. (Canceled)

58. (Currently Amended) The vector of claim 19 wherein expression of the gene product in the host cell does not rely on splicing the recombinant adeno-associated viral vector comprising at least one cis-acting heterologous transcriptional regulatory element and the second recombinant adeno-associated viral vector do not contain a heterologous splice site.

59. (Currently Amended) The composition of claim 1 wherein the two rAAVs are not splicing vectors expression of the gene product in the host cell does not rely on splicing.

60. (New) A first rAAV comprising a first recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises a promoter; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV, wherein the first rAAV does not encode a protein; and

a second rAAV comprising a second recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the promoter in the first rAAV regulates transcriptional expression of the gene product encoded by the open reading frame in the second rAAV in a host cell contacted with the first and second rAAVs.

61. (New) A first rAAV comprising a first recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises a promoter; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV; and

a second rAAV comprising a second recombinant DNA molecule comprising linked: a first DNA segment comprising a 5'-inverted terminal repeat of AAV; a second DNA segment which comprises an entire open reading frame for a therapeutic gene product; and a third DNA segment comprising a 3'-inverted terminal repeat of AAV,

wherein the second rAAV does not comprise a heterologous promoter 5' to the open reading frame.

62. (New) The first rAAV of claim 60 wherein the second DNA segment of the first rAAV further comprises an enhancer.

63. (New) A composition comprising the first rAAV and the second rAAV of claim 60.

64. (New) The composition of claim 63 further comprising a pharmaceutically acceptable carrier.

65. (New) The first rAAV of claim 61 wherein the second DNA segment of the first rAAV further comprises an enhancer.

66. (New) A composition comprising the first rAAV and the second rAAV of claim 61.
67. (New) The composition of claim 66 further comprising a pharmaceutically acceptable carrier.